AZOLLA: ITS DECOMPOSITION AND NITROGEN AVAILABILITY TO RICE (*ORYZA SATIVA*) UNDER PADDY SOIL CONDITIONS

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CHAPTER I

GENERAL INTRODUCTION

Rice is a major source of nutrition for over 60 percent of the world's population (Janick et al., 1974). In the tropics, it is the food crop produced in the largest quantity and occupies the largest area of land devoted to cultivation (Sanchez, 1976). In many of the regions where rice is grown inorganic nitrogen fertilizers are often unavailable, especially to the small subsistence farmer or sharecropper whose economic resources are limited. The high and rising cost of fossil fuels used in the manufacture of inorganic nitrogen fertilizers has contributed to their high cost. The dependency of many tropical countries on the importation of nitrogen fertilizers has resulted in erratic availability and, consequently, even higher costs for these fertilizers (Lappe and Collins, 1978).

The problem of fertilizer cost and availability to the small farmer has been compounded by the introduction of the short statured, japonica varieties of rice, developed as part of the "green revolution". The yield potentials of these new varieties is significantly higher than that of the traditional, indica varieties. However, these high yields are dependent upon a number of increased inputs, a major one being nitrogen fertilizers.

One response to the increased requirement of rice for nitrogenous fertilizers is to adopt technologies involving the use of nitrogen fixing crops used as green manures. These crops can be grown in the off season or as part of a crop rotation. They are not employed for their direct nutritional value. Instead, they are plowed into the soil where they undergo decomposition, thus supplying the following crop with some or all of the nitrogen needed to attain the increased yields.

A number of factors influence the availability of nitrogen from a green manure. These factors can be classified under two headings: (1) those factors affecting nitrogen accumulation and growth of the green manure crop, and (2) those factors affecting nitrogen mineralization and subsequent nitrogen transformations once the green manure is incorporated into the soil. The first group of factors determine the quality and quantity of green manure obtained (Lohnis, 1925). This can be optimized through the use of genetic selection, measures to control disease and insect attack, and other agronomic practices which ensure high rates of growth and nitrogen fixation of the green manure crop. The second group of factors determine the rate at which nitrogen will be made available to the crop plant from the decomposition of the green manure (Waksman and Tenney, 1927; Harmsen and van Schreven, 1955; van Schreven, 1964;

van Schreven, 1968), the soil environment into which the green manure is incorporated and the microbial flora favored by the environment (Waksman, 1942). Use of agronomic practices which influence nitrogen transformations will affect the rate of nitrogen recovery by the crop plant.

A green manure should be selected on the basis of its ability to grow and accumulate nitrogen under the soil, climatic, and agronomic conditions required by the crop plant (Ngo, 1973). It also should not interfere with normal cultivation practices or compete in any way with the main crop (Russell, 1973). The water fern, azolla, has been used for centuries in Vietnam (Dao and Tran, 1978) and China (Liu, 1978) as a green manure in lowland or paddy rice culture. Nitrogen fixation occurs as a result of the symbiotic association between the fern, *Azolla sp.*, and the cyanobacterium, *Anabaena azollae*. It is a free floating aquatic plant with a rapid rate of vegetative growth under optimal environmental conditions. These factors make it ideally suited for use as a green manure for crops grown under flooded conditions.

When azolla is added to waterlogged soil, mineralization of the azolla nitrogen occurs. Increased yield and nitrogen uptake by rice due to the addition of azolla to the paddy soil has been reported (Watanabe et al., 1977; Rains and Talley, 1978b; Talley and Rains, 1980). Most experiments on the agronomic potential of azolla as a green manure have focused upon a single parameter (e.g., grain yield or nitrogen uptake by the rice plant). The use of azolla, however, may affect rice growth due to factors other than nitrogen availability. A green manure can also increase the organic matter content of the soil, improve soil structure (FAO, 1977), and increase the soil microbial populations (Hayashi et al., 1978). Thus, without examining a number of parameters simultaneously, it is difficult to ascertain the value of azolla as a green manure except on an empirical basis.

The experiments reported here were designed to examine the decomposition and nitrogen transformations of incorporated azolla on an integral basis. To accomplish this, two greenhouse experiments were conducted involving measurements of rice yields and nitrogen uptake as well as residual levels of inorganic soil nitrogen as affected by the addition of azolla. The first greenhouse experiment was designed to examine the nitrogen availability from azolla under both continuous flooding and intermittent flooding. Reddy and Patrick (1975) have shown that increasing the number of alternate aerobic and anaerobic periods increases the rate of organic matter decomposition and loss of soil organic nitrogren. However, the effect of agronomic practices in this soil environment should be studied since the intermittently flooded condition of the rainfed paddy field is the condition most commonly found in lowland rice culture (Sanchez, 1976).

The second greenhouse experiment involved the comparison of *A. filiculoides*, *A. mexicana* and *Leucaena leucocephala* as green manures for rice culture. The azolla species used were raised on N^{15} enriched nutrient solution which allowed for a more accurate assessment of the availability of azolla nitrogen to the rice plant.

An incubation experiment was designed to examine the decomposition rates of azolla as affected by its chemical composition. Studies by Shih et al. (1978) suggested that the relatively slow rates of azolla mineralization obtained by them may have resulted from the high content of microbially resistant legnin in the azolla root tissue. Studies by Watanabe et al. (1977) showed air dried azolla to have slower initial rates of decomposition than fresh azolla. Therefore, in the incubation experiment the rate of decomposition and nitrogen mineralization of fresh and air dried azolla and fresh azolla roots and fresh azolla fronds were compared.

This integrated examination of the transformations of azolla nitrogen should lead to a better understanding of how azolla works as a green manure. In addition, it should indicate how azolla can best be used as a source of nitrogen in paddy rice culture.

CHAPTER II

LITERATURE REVIEW

A. Azolla

1. Morphology and Physiology

Azolla is a small aquatic fern with delicate bilobed leaves borne alternately on a short, floating stem. The lower leaf lobe, or aquatic lobe (Nguyen, 1930), lacks chlorophyll and is colorless. Leaf cavities within the chlorophyll containing upper leaf lobes are occupied by the cyanobacterium Anabaena azollae, under all natural conditions (Moore, 1969). The alga, closely associated with the apical meristem of the fern, grows in unison with the fern (Ashton and Walmsley, 1976) and is present during all stages of frond development.

The alga and fern are symbionts with a relationship functionally similar to the legume-rhizobium association (Holst, 1978). The azolla provides spatial protection for the cynabacterium while the anabaena fixes atmospheric nitrogen for the fern (Saubert, 1949; Peters and Mayne, 1974). Photosynthates may be transferred from the azolla to the anabaena since a high correlation has been observed between the growth rate of the fern and the rate of nitrogen fixation (acetylene reduction) by the alga (Ashton and Walmsley, 1976).

The azolla also appears to protect the anabaena's nitrogenase enzyme against inactivation by fixed nitrogen in the media. When the association is grown in media containing either nitrate or urea for a month or less the capacity for nitrogen fixation by the alga is decreased but not suppressed (Peters and Mayne, 1974; Peters, 1977). However, prolonged growth (six to seven months) of the association on a source of combined nitrogen does result in decreased rates of nitrogenase activity although the reason for this decreased activity has not been ascertained (Peters and Mayne, 1974).

The potential rate of nitrogen accumulation by the azolla-anabaena association has been reported as ranging from 310 to 500 kg N/ha year (Becking, 1978). Environmental and nutritional factors affecting the rate of growth and nitrogen fixation by the association determine the nitrogen accumulation obtained. Optimal temperatures for azolla growth and biomass production are in the 20° to 28° C range (Tran and Dao, 1973; FAO, 1977). Maximal rates of acetylene reduction for A. filiculoides are obtained when temperatures within the azolla mat are in the 30° to 40° C range (Rains and Talley, 1978b). However, Toia et al. (1979) found optimal growth and acetylene reduction activity for several species of azolla including A. filiculoides to occur at 25° to 30° C.

All essential nutrients required by higher plants other than fixed nitrogen must be available for the uninhibited growth of the association. The effects of nutrient deficiencies on the growth and nitrogen fixing capabilities of the association have been discussed by Ashton and Walmsley (1976), Watanabe et al. (1977), and Becking (1978). Iron and phosphorous were found by Talley et al. (1977) to be the nutrients most likely to limit azolla growth.

Talley et al. (1977) observed significantly greater azolla biomass production when either A. filiculoides or A. mexicana was fertilized with both phosphorous and iron compared to fertilization with phosphorous only. The critical concentration of iron in water for azolla growth was found by Rains and Talley (1978b) to be 20 µg Fe/liter. Studies by Olsen (1970) showed that azolla used iron in the ferrous form more efficiently than in the ferric form. Azolla grows at pH values ranging from 4.0 to 10.0 (Becking, 1978); optimal growth is at a pH of 5.4 to 5.5 (Saubert, 1949; Watanabe et al., 1977) where the ferrous form of iron predominates.

Phosphorous applications are usually necessary for optimal growth and nitrogen fixation by azolla. When grown under conditions of high temperature and light and low phosphorous availability, azolla fronds become stunted and develop a reddish-purple color due to increased levels of anthocyanin (Lumpkin, 1978). Phosphorous deficiency results in low rates of acetylene reduction (Espinas and Watanabe, 1976) and nitrogen accumulation (Lumpkin, 1978). Recommended rates of phosphorous fertilization are 2 to 4 kg P/ha every five days for *A. pinnata* in Vietnam (Tran and Dao, 1973) or 26 kg P/ha for a single fallow season crop of *A. filiculoides* in California (Rains and Talley, 1978b). For each kilogram of phosphorous applied, 5 kg of nitrogen are assimilated in the azolla biomass (Talley et al., 1977). Vietnamese workers noted that in situations where phosphorous fertilizers were more readily available than nitrogen fertilizers, azolla can be used to convert phosphorous into assimilated nitrogen (Tran and Dao, 1973).

Azolla has a moisture content of 90 percent to 94 percent (Saubert, 1949; Brotonegoro and Abdulkadir, 1976) and a nitrogen content of 3 percent to 6 percent on a dry weight basis (Espinas and Watanabe, 1976; Peters, 1977). An azolla mat can double its biomass in three to six days under favorable conditions (Peters, 1977). Maximum levels of biomass production are dependent on the species of azolla examined. *A. filiculoides* forms thick, multilayered mats with a biomass as high as 2750 kg dry weight/ha, representing 105 kg N/ha (Talley et al., 1977). Starting from an inoculum of *A. filiculoides* equalling 1.2 kg N/ha, 52 kg N/ha were obtained in 35 days (Talley et al., 1977). In contrast, *A. mexicana* forms only a single layer of fronds over the water surface. Under optimum field conditions only 45 kg N/ha accumulates in its biomass (Rains and Talley, 1978b).

2. Use as a Green Manure

Normally, a field is innoculated with azolla at the rate of 0.1 to 0.5 kg fresh weight/m^2 (Dao and Tran, 1978; Singh, 1978). The azolla is grown for 10 to 20 days in which time 15.0 to 22.5 metric tons/ha fresh weight containing 40 to 60 kg N/ha are produced (FAO, 1977; Singh, 1978). The paddy is then drained and the azolla is incorporated into the soil. Following incorporation the field is reflooded and the rice is transplanted. Azolla may again be inoculated into the field and be grown simultaneously in "dual culture" with the rice. A dual culture of azolla with the growing crop allows it to serve several functions simultaneously. Natural decomposition of azolla growing in dual culture increases rice yields (Singh, 1977; Talley et al., 1977). When a dense cover of azolla forms over the water surface, growth of aquatic weeds in the paddy field is inhibited (Braemer, 1927; Rains and Talley, 1978b). The presence of an azolla mat can also effectivley reduce evaporation of floodwater from the paddy (Karamyshev, 1957).

For maximum benefit from the use of a dual culture, the Chinese recommend planting rice in "double-narrow rows" in which the space between plants in the wide row is 53 to 66 cm and only 6.5 cm in the narrow row (Liu, 1978), and incorporating the growing azolla into the soil two or three times during the rice growing season (FAO, 1977). The use of double-narrow rows allows for prolonged azolla propagation in the paddy without the azolla interfering with rice growth (Liu, 1978). Incorporation of azolla during the rice growing season, which is done by hand, provides the rice with two to three times the nitrogen supplied by a single initial incorporation of azolla (FAO, 1977).

Field and greenhouse experiments have been used to assess the effectiveness of azolla as a green manure. In India, Singh (1978) showed that azolla applications containing 12 and 24 metric tons fresh weight/ha (approximately 30 kg N/ha and 60 kg N/ha, respectively) increased rice yields 9 percent and 15 percent over unfertilized controls. Ammonium sulfate applications at 20 kg N/ha, 40 kg N/ha and 60 kg N/ha produced rice yields 6.1 percent, 12.5 percent, and 19.4 percent, respectively, greater than the unfertilized controls. In California, where *A. filiculoides* equivalent to 60 kg N/ha was incorporated into the soil prior to rice planting and an additional 30 kg N/ha was produced by the azolla in dual culture with the rice, yields of 4.0 metric tons of grain/ha were obtained. This was a 308 percent increase over the unfertilized control treatment (Talley et al., 1977). The increases in crop yields when *A. filiculoides* and *A. mexicana* were used in dual culture only in California (Talley et al., 1977) were 300 and 850 kg/ha, respectively, more than the unfertilized control.

When *A. pinnata* was incorporated into the soil prior to rice transplant (Singh, 1977) a threefold increase in greenhouse rice grain yields was obtained. Unincorporated dual cultures of azolla with rice doubled rice yields relative to unfertilized controls.

Rice grain yields were increased 12 percent over unfertilized controls at the International Rice Research Institute (IRRI) when phosphorous fertilized *A. pinnata* was incorporated into the soil after growing in dual culture with the rice for 39 days (Watanabe et al., 1977).

Rains and Talley (1978a) reported that Calrose rice grain yields for field plots fertilized with *A. filiculoides* were equivalent to 40 kg N/ha N and 50 kg N/ha as $(NH_4)_2SO_4$. However, greenhouse experiments at IRRI (Watanabe et al., 1977) indicated nitrogen from air dried *A. pinnata* to be 30 percent less available than an equivalent amount of nitrogen from $(NH_4)_2SO_4$. In recent studies, Talley and Rains (1980) concluded that azolla nitrogen is as available as ammonical nitrogen at low levels of nitrogen application (i.e., less than 50 kg N/ha) while at higher rates ammonical nitrogen is more available than an equal amount of nitrogen as azolla. They obtained 30 percent lower rice grain yields from the application of 90 kg N/ha as air dried *A. filiculoides* than from an equal amount of nitrogen as $(NH_4)_2SO_4$.

B. Paddy Soil

1. Chemistry

The availability of nitrogen from a green manure depends on a number of factors, including the nitrogen content of the green manure, the environment in which the green manure undergoes decomposition, and the activities of the microorganisms present in the environment. Nitrogen availability from azolla, when used as a green manure for lowland rice, will depend on the transformations azolla nitrogen undergoes in the waterlogged soil of the paddy field.

The profile of a waterlogged paddy soil is characterized by a thin aerobic layer overlaying a reduced lower layer. When the paddy is flooded, the O_2 concentration in the upper 1.0 mm to several cm of the soil will remain in equilibrium with the oxygen dissolved in the floodwater (Sanchez, 1976). The slow rate of oxygen diffusion through water and water-filled pores decreases the oxygen availability beneath this upper soil layer. Oxygen consumption by aerobic microorganisms in the upper layer exceeds the rate of oxygen diffusion through the soil profile (Russell, 1973). The enhancement of microbial activity by the presence of readily decomposable organic matter in the soil diminishes the thickness of the aerobic layer since the active soil flora will rapidly deplete oxygen from the profile (Patrick and Mahapatra, 1968).

Within hours after flooding, aerobic organisms consume the remaining oxygen in the lower soil layer and become dormant or die (Sanchez, 1976). The populations of facultative anaerobes then increases in the soil followed by those of obligate anaerobes. As the microorganisms utilize soil components and the dissimilation products of organic matter as electron acceptors in their respiratory and fermentative process, they cause the reduction of various elements in the soil (Ponnamperuma, 1977). More than one element in the soil may be reduced by the same genera of facultative anaerobic bacteria (Yoshida, 1972; Yoshida, 1978). However, it has been demonstrated that bacteria capable of reducing more than one element reduce the more oxidized element prior to the less oxidized element (Takai and Kimura, 1966; Ponnamperuma, 1972; Hammann and Ottow, 1974).

The microbial activity results in the reduction of soil components according to thermodynamic sequence, determined by the tendency of the substrate to donate electrons. The oxidation-reduction or redox potential is a quantitative measure of this tendency (Russell, 1973). Substances with high positive redox potentials will be reduced prior to substances with low positive or negative redox potentials. Therefore, nitrate with a redox potential of +430 mv (Eh7 = the redox potential adjusted to pH 7.00) will be the first soil constituent to be reduced. It will be followed by manganese (Eh7 +410 mv), ferric iron (Eh7 +130 mv), organic acids (Eh7 -180 mv), sulfates (Eh7 -200 mv), and sulfites (Eh7 -490 mv) (Sanchez, 1976).

Interactions between the rate of microbial activity and the availability of reducible substrates in the soil will determine the extent to which the soil becomes reduced following submergence (Russell, 1973). The presence of a readily decomposable organic matter substrate, such as azolla, in the soil will increase the rate of microbial activity (Russell, 1973). Thus the rate of reduction of soil components will be increased (Ponnamperuma, 1972). Due to the abundance of iron in soil relative to other reducible substrates, the conversion of ferric iron to the ferrous form is usually the most important reduction reaction in paddy soil (Russell, 1973; Sanchez, 1976).

After flooding a paddy soil, the pH initially decreases followed by an increase after which it stabilizes within a few weeks at a pH of 6.7 to 7.2 (Ponnaperuma, 1972). The initial decrease in the soil pH probably results from an accumulation of CO_2 in the soil due to the activities of soil microorganisms (Ponnamperuma, 1972). The increase in soil pH occurs concurrently with the reduction of soil components since the major reduction reactions occurring in the paddy soil involve the consumption of H+ ions (Ponnamperuma, 1972).

2. Microbiology

Microbially facilitated nitrogen transformations which may occur when a green manure is incorporated into a soil include ammonification, nitrification, and denitrification. Ammonification involves the hydroloysis and decarboxylation of proteins to amino acids and amines by microbial enzymes which are further broken down by microbial transaminase and deaminase systems to form ammonia (Harmsen and van Schreven, 1955). Under aerobic conditions this transformation, resulting in the mineralization of organic nitrogen, is carried out by a heterogeneous group of aerobic microorganisms including bacteria, fungi, and actinomycetes (Alexander, 1977).

Anaerobic bacteria are primarily responsible for organic matter decomposition when oxygen is lacking (Alexander, 1977). Hiura et al. (1977), incubated soils under submerged conditions and found a close correlation between the amount of nitrogren mineralized and the number of gram positive rod shaped bacteria. Based on this finding, they suggested that clostridia and actinomyces may be primarily responsible for the mineralization of organic nitrogen. The degradation of organic matter under anaerobic conditions proceeds optimally at a slightly alkaline pH of 7.5 (Acharya, 1935a). Anaerobic decomposition is incomplete resulting in the production of NH_4, amines, CO_2, organic acids, mercaptans, and H_2S as microbial end products.

Under anaerobic conditions the mineralization of organic nitrogen stops at the formation of ammonium. In aerobic soils nitrifying bacteria oxidize ammonium to nitrate in a two-step process known as nitrification.

When nitrate is formed, it may become unavailable for plant uptake due either to its being leached from the root zone or to its transformation into gaseous form via microbial denitrification. The bacteria involved in the process of denitrification are facultative anaerobes which use nitrate as their respiratory process electron acceptor when oxygen is absent from the environment. Thus, denitrification occurs only when the oxygen supply in the environment is insufficient to satisfy the microbial demand. However, oxygen is essential for the formation of nitrate, the substrate used in the process of denitrification (Alexander, 1977). Therefore, aerobic and anaerobic environments must be situated in close proximity either in time or place for denitrification to occur.

In flooded paddy soil an oxidized surface layer overlays a reduced profile and provides conditions favorable for the loss of nitrogen due to denitrification. Significant nitrogen losses may occur either when the soil is initially flooded due to the aerobic soil undergoing reduction or when nitrates formed in the aerobic surface layer of the continuously flooded field are leached into the anaerobic zone.

Additions of readily decomposable organic material to the soil will stimulate the activity of denitrifying bacteria. This results in denitrifying activities occurring at higher redox potentials and at an increased rate in submerged soils (Patrick and Mahapatra, 1968).

When a soil is alternately flooded (anaerobic) and drained (aerobic) as occurs in rainfed paddy fields, an ideal environment for denitrification is produced. Rapid loss of nitrogen will occur during the first period of soil submergence with a continued decrease in the nitrogen content as the number of cycles increases (Tusneem and Patrick, 1971). Reddy and Patrick (1975) found that by increasing the frequency of wet and dry cycles, the rate of denitrification is increased.

C. Decomposition of Organic Matter

The rate of microbial decomposition of organic matter when added to a soil environment is affected by the chemical composition of the plant residue (Peevy and Norman, 1948). This is influenced by the nature of the plant material at time of incorporation (Waksman, 1942). Van Schreven (1964) found that the mineralization of carbon and nitrogen was also influenced by whether the incorporated plant material was fresh or dried. Extensive studies by Waksman and Tenney (1927) on the effect of age and chemical composition of rye plants on the rate of nitrogen mineralization showed that younger plants decomposed more rapidly than older plants. This was attributed to the higher nitrogen content and lower lignin and cellulose content of the younger plants.

Nitrogen will be mineralized from organic matter only when the nitrogen content of the organic matter exceeds the metabolic needs of the microorganisms involved in its decomposition (Alexander, 1977). Harmsen and van Schreven (1955) found that investigators agree that the added organic matter must have a nitrogen content of 1.5 percent or greater (dry weight basis) in order for nitrogen mineralization to occur under aerobic conditions. Acharya (1935b) found that the nitrogen content of organic matter required for mineralization to occur was greatest under aerobic conditions, least under anaerobic conditions, and intermediate under flooded conditions.

Based on positive rice yield responses from the incorporation into waterlogged soils of straw of various nitrogen contents, Wiliams et al. (1968) concluded that a nitrogen content of less than 0.5 percent results in nitrogen immobilization while a nitrogen content of 0.6 percent or greater results in nitrogen mineralization.

Lignin is only slowly susceptible to microbial degradation under both aerobic and anaerobic conditions. Alexander (1977) noted that lignin is decomposed under anaerobiosis although the responsible microorganisms have not been identified. Lignin in soils has been shown to "fix" protein in the form of lignoprotein complexes which increase rates of nitrogen immobilization (Bremner, 1967). These complexes result in high soil organic nitrogen levels (Waksman and Tenney, 1935; Peevy and Norman, 1948).

In China, Shih et al. (1978) showed that azolla grown under favorable environmental conditions had broad thick fronds and few fine roots while azolla grown under sub-optimal conditions had thick, dense root systems. A high lignin content and wide C/N ratio was associated with the presence of a dense root system. Well grown azolla having few roots and low C/N ratios were shown to be mineralized at a faster rate resulting in their nitrogen being more readily available for rice uptake than poorly grown azolla (Shih et al., 1978).

Drying of various plant materials, including wheat and leucern, increased the C/N ratio of the plant water soluble organic fraction (van Shreven, 1964). Dried plant material was mineralized at a slower rate than fresh material of the same species during the first four weeks of incubation. However, after the lower initial rate of nitrogen mineralization from the dried material, the rate of nitrogen release from the dried material increased during the fourth to eighth week of incubation. Similar results were obtained by Watanabe et al. (1977) using incubation experiments to compare the rate of nitrogen mineralization by fresh and air dried *A. pinnata* under flooded conditions.

They found ammonification was initially more rapid from the fresh azolla. However, after six weeks of incubation, 75 percent of the dried azolla had undergone ammonification as compared to 62 percent of the fresh azolla.

The mineralization of carbon and nitrogen from A. pinnata incubated in soils under flooded and 60 percent water holding capacity conditions was compared by Brotonegoro and Abudulkadir (1978). Treatments under moist conditions initially produced higher rates of nitrogen mineralization than treatments under flooded conditions. However, the latter treatment maintained a linear increase in nitrogen accumulation after a plateau level was obtained in the former treatment. This resulted in nitrogen accumulation in treatments under flooded conditions surpassing that of treatments under moist conditions. These results are consistent with findings by Yoshino and Dei (1977). They reported a linear increase in ammonia with time when undried paddy soils were incubated under flooded and sealed conditions. A decreased rate of ammonia accumulation after the fourth week of incubation was found in soils incubated under flooded but unsealed conditions. They suggested this difference was due to nitrification and/or denitrification loss of ammonium due to the presence of an aerobic zone in the unsealed tubes.

Measurements of CO_2 evolution were used by van Schreven (1964) and Brotonegoro and Abdulkadir (1977) to estimate microbial activity as it affects the decomposition of added organic matter. The addition of A. pinnata to soils incubated under moist conditions resulted in CO_2 evolution being greatest on the fifth day of incubation. Carbon dioxide evolution from the soils amended with A. pinnata became comparable to that of the unamended soils by the twentieth day (Brotonegoro and Abulkadir, 1977). Maximum CO_2 production was obtained under 65 percent water holding capacity within the first week to two weeks of incubation (van Schreven, 1964). Microbial activity was stabilized by the fifth week of incubation. The addition of organic materials with low C/N ratios generally resulted in a greater flush of CO_2 evolution than when less nitrogenous materials were added.

CHAPTER III

THE NITROGEN MINERALIZATION AND CARBON DIOXIDE EVOLUTION OF INCUBATED AZOLLA UNDER FLOODED CONDITIONS

A. Materials and Methods

This experiment was designed to examine the rate of decomposition and nitrogen mineralization from various forms of azolla under flooded conditions. Azolla treatments included fresh and air dried whole azolla as well as azolla separated into its succulent fronds and its lignous roots.

Soil used in this experiment was the Hanalei clay (Tropi Fluvaquent, very-fine oxidic, nonacid isohyperthermic) (Foote et al., 1972). The soil was collected from the Kula Rice Station at Wailua Valley, Kauai. The plot had been in fallow for at least four years. Before this it had been used for the cultivation of paddy rice and taro.

The soil was partially depleted of nitrogen by growing rice under flooded conditions in one liter pots. The soil was separated from the larger plant roots, sieved to remove the finer roots and then dried on a greenhouse bench. After nitrogen depletion, the air dried soil contained 18.26 ppm 1 N KCl extractable NH_4 (Bremner, 1965a), and 5.69 percent organic carbon (Walkley-Black method as described by Allison, 1965).

The azolla treatments, as listed in Table 1, were placed between two 5 g air dried soil samples in a 25 x 150 mm culture tube. The soils were flooded with 15 ml of distilled water. Short shell vials (16 x 50 mm) containing 4 ml of 0.75 N NaOH were taped to the inside of each tube to absorb CO_2 evolved by microbial activities. Tubes were tightly sealed with #4 rubber stoppers and arranged in a randomized complete block design with six replicate tubes per treatment for each of eight weekly sampling periods (240 tubes in total). Two to three no soil tubes containing only 15 ml of distilled H_2O and the standard alkali CO_2 trap were included per sampling period. Tubes were incubated in the dark at $34\pm 1.5°$ C for one to eight weeks.

Once a week six tubes per treatment were removed and analyzed for levels of CO_2 and 1 N KCl extractable NH_4. Carbon dioxide production was determined according to a modification of the method described by Pramer and Schmidt (1964). For each tube analyzed, 3 ml of NaOH was removed from the short shell vial and placed in a 50 ml Erlenmeyer flask. To this sample was added 0.3 ml of 50 percent $BaCl_2$ and two to three drops of 0.1 percent phenolphthalein. The solution was titrated to a white end point with 0.1 N HCl. The CO_2 production was calculated by: $CO_2 = (B - V)NE$ where: V = volume of 0.1 N HCl used in the titration; B = volume of 0.1 N HCl used to titrate the no soil blank; N = the normality of the HCl (0.1 N); E = 22, the equivalent weignt of CO_2.

Table 1

Treatments Used in the Incubation Experiment to Evaluate the Carbon and Nitrogen Mineralization of Various Forms of *A. Filiculoides* Under Flooded Conditions

Treatment	Nitrogen Content (Dry Weight Basis)	Nitrogen Added	Carbon Added*
	--- % ----	-µg/g Soil-	-mg/g Soil-
Fresh Whole Azolla	3.37	113.74	16.87
Air Dried Whole Azolla	3.37	101.10	15.00
Fresh Azolla Fronds	3.48	218.56	31.41
Fresh Azolla Roots	2.61	166.39	31.87
No Azolla Control	--	--	--

*Weight of azolla carbon added assumed to be equal to one-half its dry weight.

The short shell vials were removed after CO_2 analysis from inside the culture tubes, and 15 ml of 2 N KCl were added per tube. The 2 N KCl contained 0.1 percent $HgCl_2$ to arrest microbial growth in the soil extract. The tubes were recapped, vigorously shaken by hand and the soil solution was filtered through Whatman #1 filter paper. Saturated NAOM was added to aliquots of the soil filtrate containing 20 to 40 µg N and the ammonia was steam distilled into 100 ml volumetric flasks with a micro-Kjeldahl apparatus (Bremner, 1965a). The NH_4^+ content of the distillate was determined colorimetrically according to the method described by Mitchell (1972).

B. Results

Results from this experiment were confounded by the unequal amounts of nitrogen and carbon added to the different treatments. This was caused by a difference in the moisture content of the materials used. By calculating the percent carbon and nitrogen mineralization due to the addition of the azolla treatments, this confounding factor was partially corrected.

The high level of organic matter in the soil resulted in high initial levels of nitrogen mineralization after flooding the air dried soil as seen in Table 2. The large amount of organic carbon also provided substrate for high levels of CO_2 production throughout the experimental period as seen in Table 3.

Table 2

Average Values for Nitrogen Mineralization after One to Eight Weeks of Incubation (Sampling Periods 1 through 8)

Incubation Time	Treatment*	NH_4 Mineralization Cumulative	Weekly
-- Weeks --		--------- ppm	----------
1	W	68.0 a**	68.0 a
	D	60.8 b	60.8 b
	R	67.1 a	67.1 ab
	F	64.9 ab	64.9 ab
	B	66.0 ab	66.0 ab
2	W	97.9	29.8
	D	88.3	27.5
	R	92.0	24.9
	F	96.5	31.4
	B	90.3	24.3
3	W	112.3 b	14.4 b
	D	107.1 b	18.8 b
	R	109.4 b	17.4 b
	F	128.5 a	32.1 a
	B	103.7 b	13.4 b
4	W	153.8 b	41.5 a
	D	116.5 cd	9.4 b
	R	118.8 c	9.4 b
	F	166.0 a	37.5 a
	B	105.8 d	2.1 b
5	W	145.7 b	-8.1
	D	112.7 cd	-3.9
	R	119.9 c	1.0
	F	168.8 a	2.9
	B	98.2 d	-7.6
6	W	131.0 b	-14.7 c
	D	120.5 bc	7.9 ab
	R	109.0 c	-10.8 bc
	F	169.8 a	1.0 abc
	B	109.6 c	11.4 a

Table 2 (Continued)

Average Values for Nitrogen Mineralization after One to Eight Weeks of Incubation (Sampling Periods 1 through 8)

Incubation Time	Treatment*	NH_4 Mineralization Cumulative	Weekly	Increase Over Control
-- Weeks --		------- ppm	------	
7	W	137.4 b	6.4 a	
	D	104.7 cd	-15.8 b	
	R	113.3 c	4.3 a	
	F	159.9 a	-10.0 b	
	B	98.0 d	-11.6 b	
8	W	150.8 b	13.4	
	D	130.6 c	25.9	
	R	137.0 bc	23.7	
	F	178.0 a	18.1	
	B	108.9 d	11.0	
				-- % --
Averages for All Sampling Periods by Treatment	W	124.6 b	18.8 ab	28.80 a
	D	105.2 c	16.3 ab	7.53 b
	R	108.3 c	17.1 ab	6.47 b
	F	141.5 a	22.3 a	20.13 a
	B	97.6 d	13.6 b	--
Averages of All Treatments by Sampling Period				
Sampling Period	1	65.4 f	65.3 a	0.77 e
Sampling Period	2	93.0 e	27.6 b	2.11 de
Sampling Period	3	112.7 d	19.3 c	6.40 d
Sampling Period	4	132.2 b	20.0 c	22.05 ab
Sampling Period	5	129.1 bc	-3.1 d	25.40 a
Sampling Period	6	128.0 bc	-1.0 d	14.24 c
Sampling Period	7	122.2 c	-5.3 d	19.73 b
Sampling Period	8	141.1 a	18.4 c	26.70 a

*Treatments are: fresh whole azolla (W); air dried whole azolla (D); fresh azolla roots (R); fresh azolla fronds (F); and no azolla control (B).

**Treatments followed by the same letter are not significantly different based on BLSD P = 0.01.

Table 3

Average Values for CO_2 Evolution and Carbon Mineralization after One to Eight Weeks of Incubation (Sampling Periods 1 through 8)

Incubation Time	Treatment*	Cumulative CO_2 Evolution	Increase in Carbon Mineralization Over Control
-- Weeks --		-- mg/g soil -	-- % --
1	W	7.74 ab**	2.05
	D	7.09 ab	1.14
	R	6.87 ab	2.35
	F	7.86 a	1.21
	B	6.47 b	--
2	W	17.06 b	3.15 a
	D	14.73 c	-0.69 b
	R	17.04 b	1.66 a
	F	19.05 a	3.42 a
	B	15.11 c	--
3	W	22.78 ab	3.68
	D	21.43 bc	1.67
	R	22.75 ab	1.92
	F	23.37 a	2.48
	B	20.51 c	--
4	W	25.10 b	3.98
	D	25.83 b	5.81
	R	27.66 a	4.30
	F	26.87 ab	3.67
	B	22.64 c	--
5	W	29.71 a	6.25 a
	D	28.12 a	4.13 ab
	R	28.14 a	1.96 b
	F	28.83 a	2.59 b
	B	25.84 b	--
6	W	30.14 ab	0.37
	D	29.57 b	-0.59
	R	31.45 ab	1.33
	F	31.93 a	1.76
	B	29.90 ab	--

Table 3 (Continued)

Average Values for CO_2 Evolution and Carbon Mineralization after One to Eight Weeks of Incubation (Sampling Periods 1 through 8)

Incubation Time	Treatment*	Cumulative CO_2 Evolution	Increase in Carbon Mineralization Over Control
-- Weeks --		-- mg/g soil -	-- % --
7	W	33.61 abc	-0.91 ab
	D	32.31 bc	-3.38 b
	R	35.11 a	0.81 a
	F	34.46 ab	0.25 ab
	B	31.57 c	--
8	W	40.31 a	6.50 a
	D	37.51 bc	2.02 c
	R	37.99 b	4.57 b
	F	41.56 a	1.44 c
	B	36.30 c	--
Averages for All Sampling Periods by Treatment	W	25.80 b	3.13 a
	D	24.57 c	1.28 c
	R	25.88 b	1.72 bc
	F	26.74 a	2.50 ab
Averages of Treatment Values by Sampling Period			
Sampling Period	1	7.2 h	1.19 c
Sampling Period	2	16.6 g	1.88 bc
Sampling Period	3	22.2 f	2.44 b
Sampling Period	4	25.6 e	4.44 a
Sampling Period	5	28.1 d	3.73 a
Sampling Period	6	30.6 c	0.72 c
Sampling Period	7	33.4 b	-0.81 d
Sampling Period	8	38.7 a	3.68 a

*Treatments are: fresh whole azolla (W); air dried whole azolla (D); fresh azolla roots (R); fresh azolla fronds (F); and no azolla control (B).

**Treatments followed by the same letter are not statistically different based on BLSD P = 0.01.

Nitrogen added to soil as azolla was mineralized slowly during the first three weeks of incubation (Table 2 and Figure 1). A flush of nitrogen mineralization was observed between the third and fourth weeks of incubation. The whole fresh azolla and the fresh azolla frond treatments showed increases in NH_4^+ production significantly greater than the other treatments at the fourth week of incubation. No significant increases in the mineralization of nitrogen occurred between the fourth and seventh sampling periods. However, by the final sampling period the amount of nitrogen mineralized from the air dried azolla was not significantly different from the amount of nitrogen mineralized from the fresh whole azolla. Throughout the incubation period, cumulative nitrogen mineralization of fresh whole azolla and fresh azolla fronds were significantly greater than that of air dried azolla or fresh azolla roots.

A flush of CO_2 (Table 3 and Figure 2) was produced during the first two weeks of incubation. Thereafter, CO_2 production decreased with time except for a small significant increase between the seventh and eighth sampling period. This late flush of CO_2 production was greatest for the fresh whole azolla and the fresh azolla fronds treatments. Rates of carbon mineralization from the treatments throughout much of the experimental period were not significantly different. However, averaged values for CO_2 production per sampling period were significantly greater for treatments

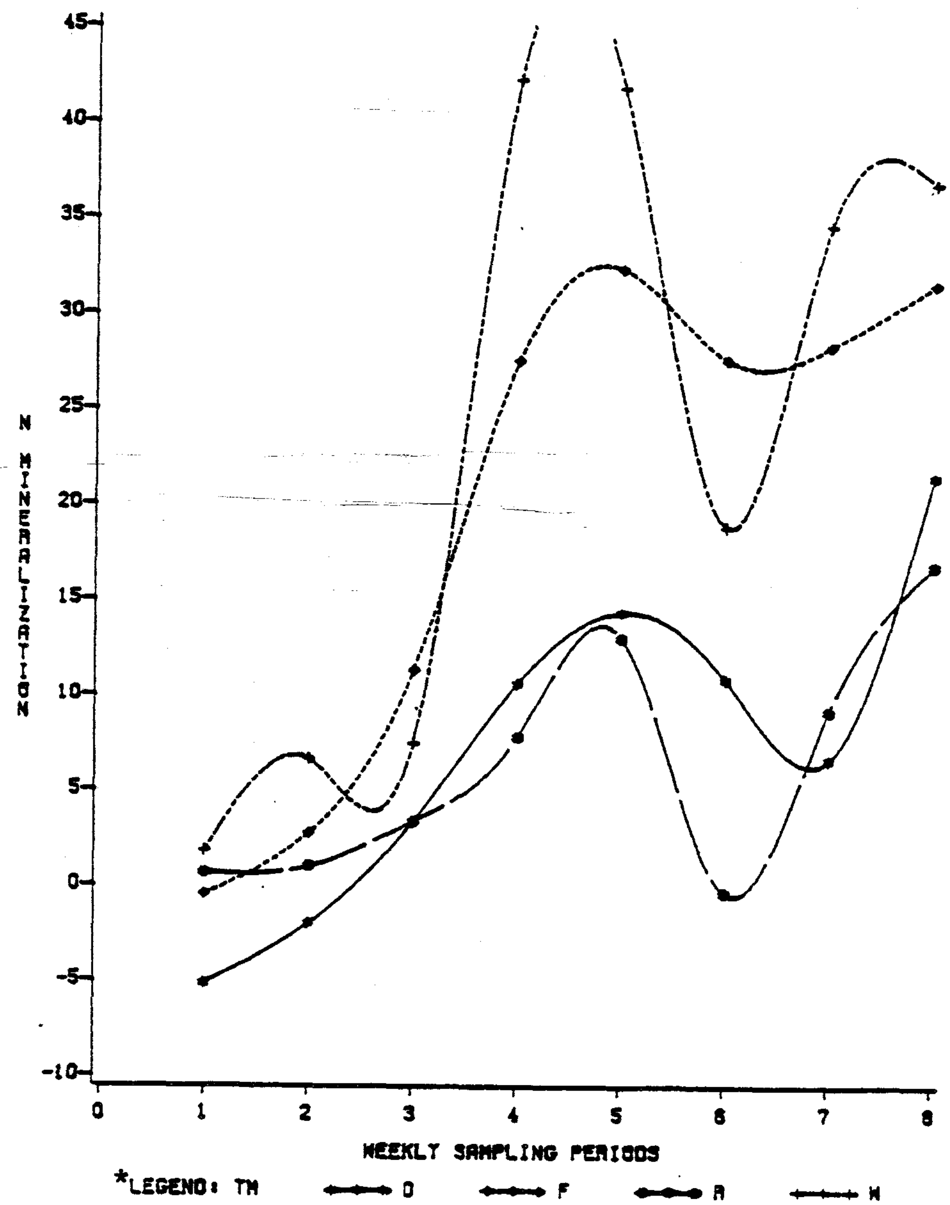

Figure 1. The Percent of Incorporated Azolla Nitrogen Mineralized in Excess of Control by Weekly Sampling Periods.

*Treatments from the incubation experiment are: D--whole air dried azolla; F--fresh azolla fronds; R--fresh azolla roots; and W--whole fresh azolla.

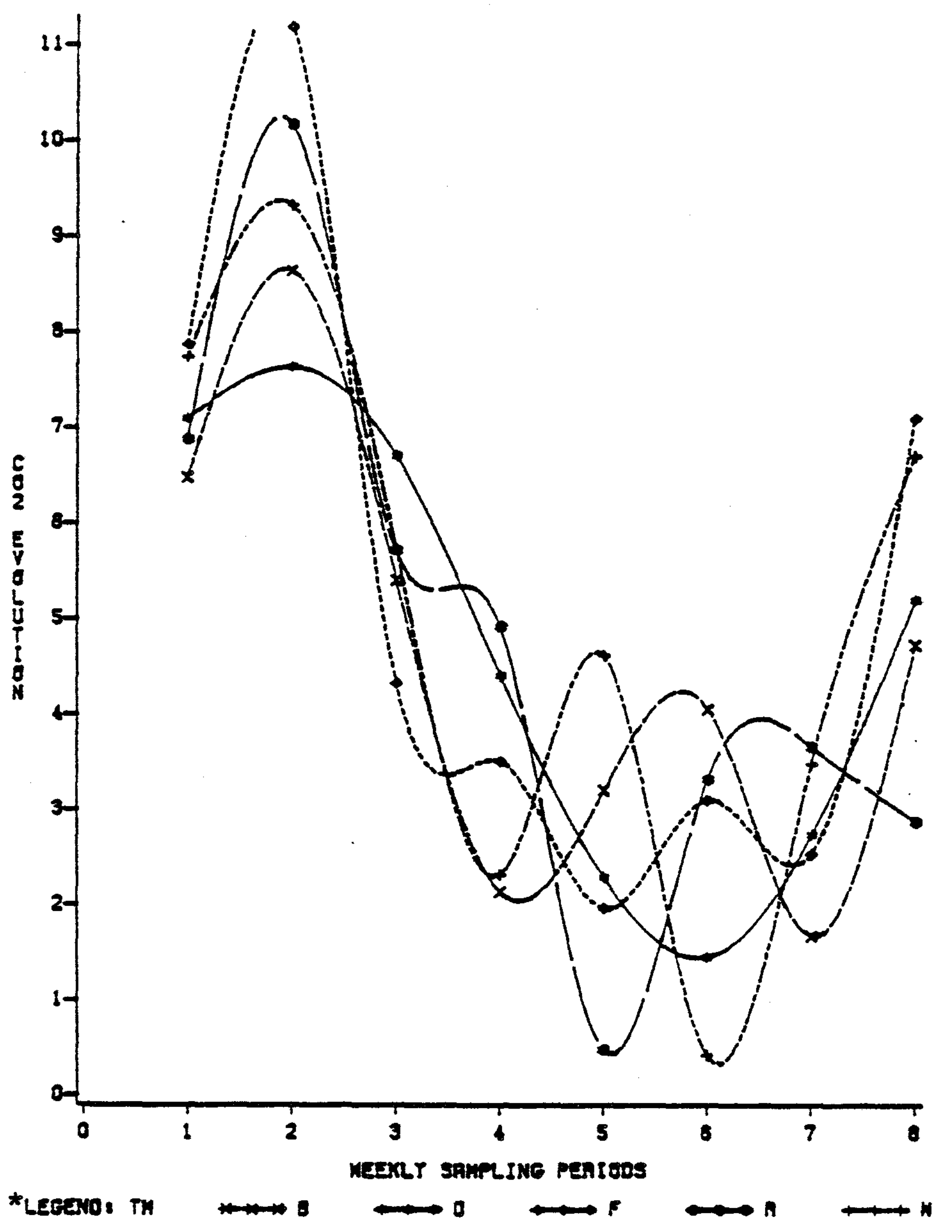

Figure 2. The Evolution of Carbon Dioxide (mg/g Soil) During Each Week of Incubation.

*Treatments from the incubation experiment are: B--no azolla control; D--whole air dried azolla; F--fresh azolla fronds; R--fresh azolla roots; and W--whole fresh azolla.

containing fresh azolla fronds than from treatments containing air dried azolla. Only 6.5 percent of the carbon added as azolla was mineralized from any treatment.

C. Discussion

The plateau in nitrogen mineralization from azolla after four weeks of incubation obtained in this study contrasts with results of Watanabe et al. (1977) who obtained linear rates of azolla mineralization throughout the course of an eight week incubation experiment. An accumulation of organic acids causing a decrease in soil pH may have resulted in a repression of further decomposition and nitrogen mineralization activities in these incubation studies. Acharya (1935a) reported that microbial decomposition of rice straw under anaerobic conditions proceeded in two distinct stages. The first stage involved the formation of organic acids while the second stage involved the subsequent conversion of the organic acids to CO_2 and CH_4. Continued rapid anaerobic decomposition was shown to depend upon the maintenance of a slightly alkaline pH since the second stage of this process is pH sensitive, occurring optimally at a pH of 7.5.

A maximum of 42.2 percent of the fresh azolla nitrogen and 21.5 percent of the air dried azolla nitrogen was mineralized in these experiments when fresh and air dried azolla treatments containing 113.7 μg N/g soil and 101.1 μg

N/g soil, respectively, were incubated under flooded conditions. In contrast, when Watanabe et al. (1977) incubated fresh and air dried A. pinnata containing 497 μg N/g soil and 273 μg N/g soil, respectively, 73 percent to 75 percent of the azolla nitrogen was mineralized after eight weeks of incubation. However, mineralization rates for fresh azolla treatments of the two experiments are comparable through the first four weeks of incubation. At that time Watanabe et al. (1977) reported 59.2 percent mineralization of the fresh azolla nitrogen while 42.2 percent of the fresh azolla nitrogen was mineralized in the incubation experiments reported herein.

The more rapid initial rates of nitrogen mineralization of fresh compared to air dried A. filiculoides is in agreement with results obtained by Watanabe et al. (1977) using fresh and air dried A. pinnata and by van Schreven (1964) using various forms of organic matter with high C/N ratios. However, when van Schreven (1964) compared the rate of nitrogen mineralization from fresh and dried lucerne having a C/N ratio of 12.6, he found no significant differences. Both Watanabe et al. (1977) and van Schreven (1964) reported that added organic materials which have low initial rates of nitrogen mineralization show increased rates of nitrogen mineralization between the fourth and the eighth weeks of incubation. The amounts of accumulated NH_4 from both fresh and air dried treatments thus became comparable at later stages of incubation. Similarly, in this study the amount of nitrogen mineralization from air dried azolla increased during the final week of incubation. At the eighth week the amounts of nitrogen accumulation from fresh and air dried azolla were not significantly different.

The lower rate of nitrogen mineralized from azolla roots compared to the azolla fronds is probably due to the high level of lignins in the azolla roots. Shih et al. (1978) also found that poorly grown azolla, characterized by a high lignin content, exhibited slower initial rates of nitrogen mineralization than did well grown azolla containing a lower level of lignin. Studies by Marumoto et al. (1980) indicated that the immobolization and remineralization of nitrogenous material added to soil was strongly dependent upon the degradability of the carbonaceous and nitrogenous materials within the soil. They found nitrogen accumulation in soils to which lignin was added to be significantly lower than in soils to which either glucose or cellulose was added. The effect of high lignin content on the mineralization of azolla nitrogen cannot be determined from these experiments since the lignin content of the azolla was not measured and nitrogen mineralization rate may have been confounded by the considerably lower nitrogen content of the azolla roots as compared with the azolla fronds.

The flush of CO_2 evolution indicates that microbial activity was high during the first two weeks of incubation for both treated and control soils. Brotonegoro and Abulkadir (1978) and van Schreven (1964) found maximum rates of microbial activity occurring within the first 14 days of incubation for treated soils. The flush of CO_2 evolution from the control soil in this experiment probably was due to the high carbon content of the soil used and the effect of rewetting an air dried soil. Birch (1960) demonstrated that elevated microbial respiration rates associated with rewetting a dried soil are largely dependent upon the carbon content of the soil. After the initial flush of microbial activity upon flooding the dried soil, a period of slow decomposition followed. Similar results were obtained by Birch (1958) when dried soils were moistened but not flooded.

The low levels of percentage total carbon mineralized in these experiments may have resulted from the formation of CH_4 under anaerobic conditions which was not oxidized to CO_2. Also calculations of the partial pressure of CO_2 within the incubation tubes at the final sampling period indicated that up to 0.3 mg CO_2/g soil was dissolved in the flood water. A method recently reported by Shirai and Furushi (1978) describes how accurate measurements of organic matter decomposition rates under flooded conditions were obtained by determining the CO_2 and CH_4 content in both the air and the water phase.

CHAPTER IV

A COMPARISON OF NITROGEN FROM AZOLLA AND UREA ON YIELDS AND NITROGEN UPTAKE OF RICE (*ORYZA SATIVA*) UNDER CONTINUOUS AND INTERMITTENT FLOODING

A. Materials and Methods

This experiment was designed to compare the effects of fresh azolla under continuous and intermittent flooding on rice yields and nitrogen uptake. Fresh and air dried azolla were compared with three levels of urea under continuous flooding.

Air dried Hanalei clay, as described previously, was added into eight liter plastic pots lined with 3 mil plastic bags at the rate of 7.5 kg soil per pot. The soil in the pots was flooded on 29 March 1980, and on 7 May the pots were covered with aluminum foil to prevent the growth of aquatic weeds. Flood water was drained from the pots on 3 June, the soil stirred and soil samples removed for initial nitrogen and carbon analyses. The soil contained 6.1 percent organic carbon (Walkley-Black method as described by Allison, 1965), 0.25 percent total nitrogen, and 76.6 ppm 1 N KCl extractable nitrogen (Bremner, 1965a). The *A. filiculoides* used in this experiment was grown on a nitrogen free nutrient solution of the following composition: $MgSO_4 \cdot 7H_2O$ (7.5 ppm Mg), $CaCl_2$ (15 ppm Ca), K_2SO_4 (30 ppm K),

$K_2H_2PO_4$ (10 ppm P, 12.5 ppm K), NaCl (5 ppm Na), $CoCl_2 \cdot H_2O$ (0.01 ppm Co), $CuSO_4 \cdot 5H_2O$ (0.1 ppm Cu), H_3BO_3 (0.1 ppm B), $MnCl_2 \cdot 4H_2O$ (1.0 ppm Mn), $Na_2MoO_4 \cdot 2H_2O$ (0.1 ppm Mo), $ZnSO_4 \cdot 7H_2O$ (0.5 ppm Zn) and Fe Sequestrene 138 (2.5 ppm Fe).

The treatments, as described in Table 4, were manually incorporated into the soil on 7 and 8 June 1980. The pots were placed on two greenhouse benches using a randomized complete block design with six replicates per treatment. All pots received 0.40 g P as treble super phosphate and 0.40 g K as muriate of potash incorporated into the soil simultaneously with the urea or azolla treatments. After which all pots were flooded to a depth of approximately 5 cm.

Two seedlings of IR-30 rice germinated on 25 May were transplanted into each pot on 13 June. All pots except those having a dual culture of azolla with the rice (treatments 3 and 4, Table 4) were covered with aluminum foil in which holes were cut to allow for the growth of the rice. Both aluminum foil and azolla were effective in deterring the growth of aquatic weeds.

Plants were harvested on 25 August, 75 days after transplanting. On this date, plant height, tiller number, and fresh weight were determined. It was also noted whether plants were in the vegetative or reproductive stage of growth. Plants were dried at 60° C for approximately 36 hours, dry weights were determined, and the plants were ground.

Table 4

Treatments Used in First Greenhouse Experiment to Compare the Effects of Nitrogen from Azolla and Urea on Yields and Nitrogen Uptake by Rice under Two Water Management Regimes

ɘatment Number	Treatment	Nitrogen Added	Water Management*
		—— g/pot ——	
1	Fresh azolla	0.40	Continuous flooding
2	Air-dried azolla	0.40	Continuous flooding
3	Fresh azolla + dual**	0.40 + dual** culture	Intermittent flooding
4	Fresh azolla + dual**	0.40 + dual** culture	Continuous flooding
5	Urea	0.20	Continuous flooding
6	Urea	0.40	Intermittent flooding
7	Urea	0.40	Continuous flooding
8	Urea	0.60	Continous flooding
9	Zero Nitrogen Control	—	Continous flooding
10	Zero Nitrogen Control	—	Intermittent flooding

:ontinuously flooded treatments were flooded to a depth of 4-5 cm throughout the period of rice growth. Intermittently flooded treatments were alternately flooded and dried for two week periods throughout the period of rice growth (three flooded periods and two dry periods).

Treatments 3 and 4 received 31.4 g of fresh azolla floating on the surface of the water after seedlings were transplanted (containing approximately 0.10 g N). Since the azolla died when treatment 3 was subjected to dry soil conditions, 15 g fresh azolla (containing approximately 0.05 g N) was added at the beginning of each of the two subsequent flooded periods.

Nitrogen in the ground plant material was determined by digesting 0.1 g of plant tissue in 75 ml Technicon tubes using 2 ml of H_2O_2, 5 to 7 ml technical grade sulfuric acid, 2.25 g of salt mixture (20 parts K_2SO_4: one part $FeSO_4$: two parts $CuSO_4 \cdot 5H_2O$) and two to three boiling chips. The plant material was digested at 375° C for two hours or until the digest was a light green to white in color. The digest was transferred to 100 ml volumetric flasks and brought to volume with distilled H_2O. Aliquots containing 20 to 40 µg NH_4 N were volumetrically transferred to 100 ml volumetric flasks and the nitrogen content of the aliquot was determined colorimetrically using a modification of the method described by Mitchell (1972). One to two drops of 0.1 percent methyl red solution was added to each aliquot and the solution was titrated with approximately 20 percent NaOH to a yellow end point prior to the addition of the Mitchell reagents.

Soil samples were taken from each pot following plant harvest and stored at 0° C in sealed plastic bags until they could be analyzed. After mixing the soil in each bag, 15 to 20 g of soil was weighed into 25 x 150 mm culture tubes to which 30 ml of 1 N KCl was added. The 1 N KCl contained 1.0 percent $HgCl_2$ to retard microbial growth in the soil extract. The tubes were tightly capped, vigorously shaken by hand, and the extract obtained by filtering the soil solution through Whatman #1 filter paper. The soil moisture content was obtained at the same time by drying 10 to 15 g samples of moist soil at 110° C for approximately eight hours.

Aliquots of the soil solution containing 20 to 40 g of NH were made basic by adding saturated NaOH steam distilled into 100 ml volumetric flasks. The NH_4 was then determined using a micro-Kjeldahl apparatus (Bremner, 1965a). The ammonium content of the distillate was determined colorimetrically using the method described by Mitchell (1972). Ammonium plus nitrate in the distillates were determined similarly to the ammonium content except that 0.1 ml of mineral oil and 0.25 g of Davarda's alloy was added through the side arm of the Kjeldahl flask prior to the addition of the sample.

Following harvest and soil sampling, all pots were reflooded and a ratoon crop of rice was allowed to grow. The ratoon crop was harvested on 29 October. It was dried at 60° C for approximately 36 hours. The dry weight of the plant material was recorded after which the rice was ground and its nitrogen content determined as described above.

B. Results

Results from this experiment may have been confounded by the high levels of native nitrogen in the soil as indicated by the large nitrogen uptake values for the two control treatments.

The above ground portion of the rice plant took up 15.0 percent, 37.9 percent, and 60.5 percent, respectively, of the nitrogen added as urea at the low (0.20 g N/pot, T_5 = treatment 5 from Table 4); medium (0.40 g N/pot, T_7); and high (0.60 g N/pot, T_8) rates. Only 20.0 percent, 2.1 percent, and -2.1 percent, respectively, of the nitrogen incorporated into the soil as fresh azolla (T_1), air dried azolla (T_2), and fresh azolla with dual culture (T_4), respectively, was recovered. Uptake of nitrogen from the azolla treatments were not statistically different from the urea application at 0.20 g N/pot (T_5). However, the fresh azolla treatment (T_1) was not significantly different from the urea application of 0.40 g N/pot (T_7). Under intermittent flooding, 55.8 percent of the nitrogen applied was recovered when nitrogen was added as urea (T_6). Only 27.1 percent of the nitrogen added as azolla (T_3) was recovered.

As seen in Table 6 no significant correlations between the variables measured, except for that between fresh and dry weight, were obtained. Both the urea (T_6) and the azolla treatment (T_3) under intermittent flooding conditions resulted in high levels of nitrogen uptake (Table 5) but low yields (Table 7) of the rice plants. These results may have occurred since the rice from these treatments was in the vegetative stage of growth while the rice from the continuously flooded treatments was the reproductive stage of growth at the time of harvest. High yield and nitrogen

Table 5

Average Values for Nitrogen Uptake by Rice Straw and Grain, and Residual Nitrogen at Time of Harvest (75 Days after Transplanting)

Treatment Number*	Treatment	Water Management	Nitrogen Uptake by Rice Straw and Grain: N Uptake	Nitrogen Uptake by Rice Straw and Grain: Increase Over Control**	Residual Inorganic Soil Nitrogen: NH_4+NO_3 N Uptake	Residual Inorganic Soil Nitrogen: Increase Over Control**	Available N Recovered
			- g/pot -	--- % ---	--- ppm ---	--- % ---	--- % ---
1	0.40 g N as fresh azolla	Flooded	1.00 cd	20.0 bc	22.1 a	16.1	95.2
2	0.40 g N as air dried azolla	Flooded	0.91 d	-2.1 c	14.1 bc	1.1 b	80.8
3	0.40 g N as fresh azolla + dual	Intermittent flooding	1.02 bcd	27.1 abc	10.2 de	-2.0 bc	103.2
4	0.40 g N as fresh azolla + dual	Flooded	0.93 d	2.1 c	15.1 b	1.8 b	83.5
5	0.20 g N as Urea	Flooded	0.95 d	15.0 bc	12.1 cd	-6.0 c	97.4
6	0.40 g N as Urea	Intermittent flooding	1.14 b	55.8 a	8.3 e	-5.6 c	106.3
7	0.40 g N as Urea	Flooded	1.07 bc	37.9 ab	13.1 bc	-0.1 bc	92.7
8	0.60 g N as Urea	Flooded	1.28 a	60.5 a	12.9 bcd	-0.1 bc	95.4
9	Zero Nitrogen Control	Flooded	0.92 d	--	13.4 bc	--	--
10	Zero Nitrogen Control	Intermittent flooding	0.915 d	--	11.3 cd	--	--

Treatments followed by the same letter are not significantly different based on BLSD P = 0.01 level.

*See Table 4 for complete treatment descriptions.

**Treatments 1, 2, 4, 5, 7, and 8 were compared with treatment 9. Treatments 3 and 6 were compared with treatment 10.

Table 6

Correlation Coefficients between Variables Measured in First Greenhouse Experiment

	Fresh Weight	Dry Weight	N UPTAKE Plant Crop	N UPTAKE Ratoon Crop	Soil Nitrogen
Dry Weight	0.925* 0.874** 0.681***				
N Uptake by Plant Crop	0.066 0.393 0.190	0.097 0.257 0.121			
N Uptake by Ratoon Crop	N.A.	N.A.	0.066 0.016 0.110		
Soil	0.342 -0.027 -0.127	0.304 -0.181 -0.308	-0.117 -0.016 -0.325	-0.117 -9.091 -0.304	
N Added	0.466 0.551 0.329	0.347 0.401 0.112	0.518 0.529 0.567	-0.088 0.125 0.107	0.110 -0.127 -0.409

*r value for all treatments
**r value for continuously flooded treatments (1, 2, 4, 5, 7, 8, 9)
***r value for intermittently flooded treatments (3, 6, 10)

Table 7

Yield Above Ground Portion of Rice Plants from the First Greenhouse Experiment (Dry Weight Basis) Measured 75 Days after Transplant

Treatment Number*	Treatment	Water Management	Plant Moisture Content	Rice Yield: Dry Weight Basis	Rice Yield: Increase Over Control**
			------- % ------	-- g/pot ---	---- % -----
1	0.40 g N as fresh azolla	Flooded	27.1 a	106.8 ab	15.7 ab
2	0.40 g N as air dried azolla	Flooded	25.6 abc	95.0 bc	2.9 bc
3	0.40 g N as fresh azolla + dual culture	Intermittently flooded	22.4 d	65.2 d	-3.0 c
4	0.40 g N as fresh azolla + dual culture	Flooded	27.0 a	109.0 a	18.0 ab
5	0.20 g N as Urea	Flooded	27.6 a	108.5 a	17.5 ab
6	0.40 g N as Urea	Intermittently flooded	24.0	72.2 d	7.4 bc
7	0.40 g N as Urea	Flooded	25.7 abc	108.0 a	17.0 ab
8	0.60 g N as Urea	Flooded	26.7 ab	118.0 a	27.8 a
9	Zero Nitrogen Control	Flooded	27.9 a	92.3 c	--
10	Zero Nitrogen Control	Intermittently flooded	24.3 bcd	67.2 d	--

Treatments followed by the same letter are not statistically different based on BLSD P = 0.01.

*See Table 4 for complete treatment descriptions.
**Treatments 1, 2, 4, 5, 7 and 8 were compared with treatment 9, treatments 3 and 6 were compared with treatment 10.

uptake values were obtained by the application of urea at 0.40 g N/pot (T_2) and 0.60 g N/pot (T_8) (Table 5 and Table 7). However, yields from the high urea treatment (T_8) were not significantly different from the fresh azolla treatment with the dual culture (T_4) which had a very low nitrogen uptake value. The incorporation of fresh azolla (T_1) resulted in increased nitrogen uptake and yields compared to the incorporation of air dried azolla (T_2). However, these differences were not statistically significant.

With the addition of fresh azolla (T_1), residual soil nitrogen values were significantly greater than that of all the other treatments (Table 5). All continuously flooded azolla treatments (T_1, T_2, T_4) had soil nitrogen values greater than the continuously flooded control treatment (T_9) while all continuously flooded urea treatments (T_5, T_7, T_8) had lower levels of soil nitrogen than the control (T_9). Under intermittent flooding higher levels of soil nitrogen were obtained from the azolla treatment (T_3) than from the urea treatment (T_6). However, both treatments had soil nitrogen levels lower than the control (T_{10}), although only the values obtained from the urea treatments (T_6) were significantly lower.

Recovery of added nitrogen from all sources; plant crop uptake, ratoon crop uptake, and soil nitrogen, indicated a greater than 100 percent recovery rate for the intermittently flooded treatments (T_3, T_6) (Table 5). The addition of air dried azolla (T_2) and of fresh azolla with dual culture (T_4) under continuous flooding resulted in significantly lower levels of nitrogen recovery than the other azolla treatments.

C. Discussion

1. High Carbon and Nitrogen Content of Soil

The use of an air dried soil with a high carbon content accounted for the high initial levels of soil nitrogen and the subsequent high yields and nitrogen uptake from the no nitrogen treatments in this experiment. Birch (1960) reported high levels of nitrogen mineralization occurred when an air dried soil was rewetted. The magnitude of the increase in inorganic soil nitrogen was found by him to be largely a function of the soil carbon content. Similar results were reported by Ventura and Watanabe (1978). They found that an alternate wet and dry cycle of 20 days duration prior to transplanting rice increased nitrogen uptake by the rice plants.

2. Continuous versus Intermittent Flooding

Nitrogen uptake for those treatments subjected to intermittent flooding was not significantly different from corresponding treatments under continuous flooding. However, the yields for treatments under intermittent flooding were significantly lower than for the same treatment under continuous flooding. The lower yields were due to a slower rate of maturation for the rice grown under intermittent flooding compared to continuous flooding. Their slower rate of maturation was probably due to water deficiencies which may have occurred during the periods of dry soil conditions and/or from higher levels of nitrogen in the plants. De Datta et al. (1973) reported rice yields decreased 1.0 t/ha when soil moisture tension in the soil was maintained at 15 centibars or more. It is unlkely that plants subjected to intermittent flooding were affected detrimentally by changes in pH or redox potential due to changes in soil water conditions. Measurements of pH taken during the first dry cycle showed no differences between the pH of the flooded and the dried soils.

Soil nitrogen levels for the intermittently flooded treatments were lower than those of all the continuously flooded treatments including the control. The low levels of soil nitrogen obtained in the intermittently flooded treatments were due either to the higher levels of soil ntirogen taken up by the rice plants (see Table 5) or possibly from denitrification losses which might have occurred when the dry soil was reflooded. Reddy and Patrick (1974) reported rates of decomposition of organic matter and losses of total nitrogen from the soil increased as the number of alternating aerobic and anaerobic soil cycles increased. Results from this experiment are in agreement with those of van Schreven (1968). He indicated that soil drying at temperatures below 35° C did not stimulate the mineralization of incorporated fresh plant material.

3. A Comparison of Azolla and Ammonical Fertilizers and Rates of Nitrogen Fertilization

The higher nitrogen uptake values obtained from the urea treatments compared to the incorporation of fresh azolla under continuous flooding are similar to the results obtained by Watanabe et al. (1977) when the nitrogen availability from $(NH_4)_2SO_4$ and air dried A. pinnata was compared. However, in this experiment nitrogen availability from air dried A. filiculoides was considerably lower than from fresh A. filiculoides. These results are consistent with those obtained by van Schreven (1968) using various fresh and air dried plant materials. Differences in the absolute levels of nitrogen recovery as measured by nitrogen uptake by the rice plants reported in this experiment compared to those of Watanabe et al. (1977) may be due to differences in the age of the plant at sampling. Yoneyama and Yoshida (1977) indicated uptake of $(NH_4)_2SO_4$ by rice in pot experiments was at a maximum during the first 40 days of rice growth while maximum nitrogen recovery from straw was obtained between 60 and 90 days. Plants in the experiment reported herein were harvested at 75 days or during the time when maximum nitrogen uptake of azolla nitrogen was probably occurring. The plants used by Watanabe et al. (1977) were harvested at 110 days after transplanting. Significantly higher levels of inorganic soil nitrogen was found in the soil after rice harvest due to treatment with A. filiculoides without dual culture compared to the urea treatment. This result indicates that azolla mineralization had occurred which was not accounted for by rice uptake.

Rice straw and grain yields for the fresh azolla, with and without dual culture, and all levels of urea application were not significantly different. Thus, it is not possible to determine whether the availability of nitrogen from azolla is better, worse or equal to the availability of an equal application of urea. Rains and Talley (1978b) and Singh (1978) applied 40 kg N/ha and 30 kg N/ha, respectively, of azolla and $(NH_4)_2SO_4$. They obtained no significant differences in grain yields between the two sources of nitrogen. However, Talley and Rains (1980) found nitrogen from air dried A. filiculoides to be 30 percent less available than ammonical nitrogen when 90 kg N/ha was applied. The application of 0.40 g N/pot used in this experiment was approximately equal to 105 kg N/ha.

4. Azolla Incorporation versus Incorporation plus Dual Culture

The incorporated fresh azolla plus dual culture treatment produced yields not significantly different from the incorporated fresh azolla treatment without dual culture. Talley et al. (1977) obtained an increase in rice grain yields of 1230 kg/ha when A. filiculoides was used as both a green manure and as a dual culture in field studies compared to when it was employed as a green manure only. The amount of nitrogen taken up by the rice plants when azolla was used in dual culture in the experiment reported herein was significantly less than when azolla was incorporated into the soil only. These results are consistent with those recently reported by Lumpkin et al. (1981). Their studies indicated that azolla growing in dual culture with rice will lower the nitrogen availability to the rice plants. Studies by Peters and Mayne (1977) showed that azolla acetylene reduction levels decreased when the azolla was provided with a source of fixed nitrogen. These results indicated that the azolla was obtaining a percentage of its nitrogen requirement from the fixed nitrogen source.

CHAPTER V

A COMPARISON OF NITROGEN FROM TWO AZOLLA SPECIES, LEUCAENA LEUCOCEPHALA, AND UREA ON THE YIELDS AND NITROGEN UPTAKE OF RICE (ORYZA SATIVA) UNDER FLOODED CONDITIONS

A. Materials and Methods

This experiment was designed to compare the nitrogen availability from two species of azolla as fresh material, *A. filiculoides* and *A. mexicana*; air dried *A. filiculoides*; fresh *Leucaena leucocephala*; and urea under continuous flooding. Nitrogen availability was measured in terms of yields and nitrogen uptake by rice.

Hanalei clay, previously used in the experiment described in Chapter IV, was carefully removed from the rice roots. This soil was sieved to remove the smaller roots and composited in a large garbage can. Care was taken to ensure that the soil remained wet throughout the entire procedure to minimize nitrogen mineralization. Approximately 3.0 kg soil (dry weight basis) was placed into plastic pots (10 cm radius) lined with three mil plastic bags. The soil contained 19.27 ppm 1 N KCl extractable NH_4 (Bremner, 1965a) and 5.9 percent carbon (Walkley Black method as described by Allison, 1965).

Treatments, as described in Table 8, were incorporated into the soil on 30 November 1980. Two zero nitrogen treatments were included in the experimental design. One zero nitrogen treatment was run using rice seedlings grown on azolla compost as a nursery bed to determine whether the azolla compost could supply sufficient nitrogen to the rice plants during this early stage of growth to sustain later growth in a soil low in nitrogen.

The azolla used in this experiment was propagated on the azolla nutrient solution described in Chapter IV also containing 5 ml/litre 2000 ppm 5.7 atom percent $^{15}NH_4{}^{15}NO_3$. This resulted in ^{15}N labelling of the azolla and allowed for labelled nitrogen transfer studies to be undertaken.

Phosphorous in the form of treble super phosphate and potassium in the form of muriate of potash were incorporated into the soil at the rate of 0.30 g per pot of each element simultaneously with the incorporation of the treatments. The pots were placed on two greenhouse benches in a randomized complete block design with nine replicates per treatment. All pots were kept flooded to a depth of 4 to 5 cm throughout the growing period. Two rice seedlings germinated on 8 November 1980 were transplanted into each pot on 1 December 1980. All rice seedlings except those used for the second control treatment were grown until transplanted in unfertilized tap water.

On 4 December, the dual culture azolla treatments were inoculated with the appropriate species of azolla. All other pots were covered with black cloth with holes cut to allow for the growth of the rice. Since azolla not shaded by rice growth become necrotic by the second week after inoculation, 56 percent shade cloth was placed over the pots in a manner similar to the black cloth used in the other treatments. As a result, the azolla grew vigorously. Thereafter, both the azolla and the black cloth proved equally effective in preventing the growth of aquatic weeds.

The rice was harvested on 13 February 1981, 75 days after the seedlings were transplanted. At this time, plant height, tiller number, and plant fresh weight were determined. Plants were dried at 60° C for approximately 36 hours, the dry weight measured and the plant material ground. The ground plant material was digested and the nitrogen content determined as was reported in Chapter IV.

Soil samples taken from each pot at the time of harvest were sealed in plastic bags and stored at 0° C until analyzed. The 1 N KCl extractable NH_4 in the soil was determined by adding 30 ml of 1 N CKl to 25 g moist soil in a 25 x 150 mm culture tube. The 1 N KCl contained 0.1 percent $HgCl_2$ to inhibit microbial growth. The tubes were tightly sealed with #4 rubber stoppers and shaken horizontally for one hour. The soil extract was obtained by filtering the soil through Whatman #1 filter paper. The ammonium content of the extract was determined as in the previous greenhouse experiment. The soil moisture content was determined by drying 50 g of soil at 110° C for approximatey eight hours.

The roots from six pots selected at random from each treatment were washed from the soil mass under rapidly running water. The roots were dried at 60° C for approximately 60 hours. The dry weight of the roots was measured and the root material ground. The nitrogen content of the ground root material was determined as for the plant material.

The two species of azolla and the rice straw and grain from five of the nine replicates of the three incorporated azolla treatments 1, 2, 3 (Table 8) were analyzed for excess concentrations of ^{15}N. The urea treatment 6 was also analyzed as a measure of relative ^{15}N natural abundance. Dried, ground plant material at the rate of 0.75 g per tube was digested at 275° C for 14 hours in 75 ml Technicon digestion tubes using one selenium granule, 2.75 g of salt mixture (10 parts K_2SO_4: 1 part $CuSO_4 \cdot 5H_2O$), two to three boiling chips, and 8 ml technical grade H_2SO_4. Digested samples were brought to 75 ml volume with distilled H_2O. Twenty-five ml aliquots of the digests were made basic with saturated NaOH. The NH_4 was then steam distilled into 5 ml of 4 percent boric acid indicator solution. The nitrogen content of the distillates was determined by titration with 0.0429 N H_2SO_4. The acidified distillate was concentrated to approximately 3 ml on a steam plate and stored at 5° C until mass spectrometry was performed. Conversion of the concentrated NH_4 to N_2 gas and isotope analysis of the samples was performed according

Table 8

Treatments Used in the Second Greenhouse Experiment to Compare the Effects of Nitrogen from Azolla, Leucaena, and Urea on Yields and Nitrogen Uptake by Rice

Treatment Number	Treatment	Nitrogen Content	N Added	Additional Information
		--- % --	-g/pot-	
1	Fresh *A. filiculoides*	3.48 N	0.30	10 g *A. filiculoides* (0.02 g N) fresh weight was added as dual culture inoculation
2	Fresh *A. mexicana*	4.17 N	0.29	15 g *A. mexicana* (0.03 g N) fresh weight was added as dual culture inoculation
3	Air dried *A. filiculoides*	3.48 N	0.30	10 g *A. filiculoides* (0.02 g N) fresh weight was added as dual culture inoculation
4	Fresh *Leucaena leucocephala*	3.39 N	0.30	Freshly picked young leaves and petioles
5	Rice Nursery Bed Fertilization	--	--	Seedlings were grown on composted azolla containing 1500 ppm inorganic nitrogen prior to transplant
6	Urea	__	0.30	--------
7	Zero Nitrogen Control	--	--	--------

to the procedures described by Bremner (1965b) using a consolidated Electronics Corporation Model 21-103c mass spectrometer with a 21-110 electron multiplier, a resolution of 1/2500 amv (10 percent valley) and a filament at 70 e.v.

B. Results and Discussion

1. Yields

Dry weight yield data (Table 9) for the above ground portion of the rice plant revealed that urea treatments significantly outyielded all the azolla treatments. This is in agreement with results obtained by Talley and Rains (1980) for grain yields when air dried A. filiculoides and $(NH_4)_2SO_4$ were compared at nitrogen application rates of 90 kg N/ha. The 0.30 g N per pot used in this experiment was approximately equal to 80 kg N/ha.

No significant differences in grain and straw yields were observed among the three azolla treatments. All three azolla treatments had significantly higher yields than the no nitrogen treatments. When the total dry weight (above ground portion + root) was compared, the fresh A. filiculoides treatment had a total dry weight significantly greater than that of the air dried A. filiculoides treatment but not significantly different from that of the urea treatment.

2. Nitrogen Uptake

Nitrogen uptake by both the above ground portion of

Table 9

Dry Weight Yields of the Above Ground Portion and Roots of Rice from the Second Greenhouse Experiment Measured 75 Days after Transplant

Treatment Number*	Treatment	Rice Straw and Grain		Rice Roots		Rice Straw, Grain and Roots	
		Dry Weight	Increase Over Control	Dry Weight	Increase Over Control	Dry Weight	Increase Over Control
		- g/pot --	----- % -----	- g/pot --	----- % -----	- g/pot --	----- % -----
1	Fresh <u>Azolla</u> <u>filuculoides</u>	29 b**	81.9 b	22 a	123.3 a	51 ab	98.1 ab
2	Fresh <u>Azolla</u> <u>mexicana</u>	29 b	81.2 b	21 ab	106.7 ab	50 bc	90.4 bc
3	Air dried <u>Azolla</u> <u>filiculoides</u>	28 b	76.4 b	17 b	73.3 b	46 c	78.2 c
4	Fresh <u>Leucena</u> <u>leucocephalus</u>	33a	106.3 a	21 ab	110.0 ab	54 a	108.3 a
5	Rice Nursery Bed Fertilication	17 c	5.6 d	11 c	8.3 c	27 d	5.1 d
6	Urea	35 a	120.8 a	19 ab	88.3 ab	54 ab	106.4 ab
7	Zero Nitrogen Control	16 c	--	10 c	--	26 c	--

*See Table 8 for complete treatment descriptions.
**Treatments followed by the same letter are not significantly different based on BLSD P = 0.01 level.

the plant and the plant roots was significantly higher when nitrogen was applied as fresh A. filiculoides than as urea (Table 10). The low correlation found between the yield and percent nitrogen in the plant material (Table 11) probably was due to the low availability of azolla nitrogen during the early stage of rice growth. Reddy and Patrick (1976b) showed best efficiency of ammonical nitrogen utilization was obtained when nitrogen was applied early in the growing season.

Significantly higher levels of nitrogen were taken up from fresh A. filiculoides than fresh A. mexicana. However, both fresh azolla treatments resulted in significantly higher levels of nitrogen in the rice plants than the air dried A. filiculoides. The latter results are consistent with those obtained by van Schreven (1968) using various fresh and air dried organic materials. As was mentioned previously, Watanabe et al. (1977) reported nitrogen from air dried A. pinnata to be 40 percent less available than from ammonical nitrogen. In this experiment, nitrogen from the fresh A. filiculoides treatment was 12 percent more available in terms of nitrogen uptake by the above ground portion of the rice plant and 18 percent more available in terms of total plant nitrogen uptake than nitrogen from urea.

The correlation coefficient between nitrogen recovery using labelled nitrogen and nitrogen recovery using chemical

Table 10

Nitrogen Uptake by the Above Ground Portion and Roots of Rice, and Residual Inorganic Soil NH_4 Levels from Second Greenhouse Experiment 75 Days after Rice Transplant

Treatment Number*	Treatment	Nitrogen Uptake: Rice Straw and Grain: N Uptake	Nitrogen Uptake: Rice Straw and Grain: Increase Over Control	Nitrogen Uptake: Rice Straw and Grain: N^{15} Recovery**	Nitrogen Uptake: Rice Straw, Grain and Roots: N Uptake	Nitrogen Uptake: Rice Straw, Grain and Roots: Increase Over Control	Residual Inorganic Soil NH_4
		- g pot -	---- % -----	---- % -----	- g/pot -	---- % -----	------ ppm -------
1	Fresh *A. filiculoides*	0.267 a***	51.7 a	47.31	0.353 a	68.3 a	4.13 a
2	Fresh *A. mexicana*	0.237 bc	41.7 bc	40.85	0.323 b	58.3 b	3.31 ab
3	Air dried *A. filiculoides*	0.215 d	34.4 d	28.78	0.283 c	45.0 d	3.31 ab
4	Fresh *Leucena leucocephalus*	0.254 ab	47.4 ab		0.342 ab	64.8 a	3.48 ab
5	Rice Nursery Bed Fertilization	0.113 e	0.2 e		0.149 d	0.1 e	2.98 b
6	Urea	0.232 cd	40.0		0.299 c	50.3 c	2.70 b
7	Zero Nitrogen Control	0.112 e	--		0.148 d	--	2.88 b

*See Table 8 for complete treatment description.
**_A. filiculoides_ treatments had 0.3196% atom excess N^{15}. _A. mexicana_ treatments had 0.3260% atom excess N^{15}.
***Treatment followed by the same letter are not significantly different based on BLSD P = 0.01.

Table 11

Correlation Coefficients for Variables in the Second Greenhouse Experiment

	Tiller Number	Plant Fresh Weight	Plant Dry Weight	Root Dry Weight	Plant N Up	Root N Up	Total N Up
Plant Fresh Wt.	0.891**						
Plant Dry Wt.	0.843**	0.964**					
Root Dry Wt.	0.748*	0.732*	0.687*				
Plant N Uptake	0.861**	0.938**	0.892[a]	0.751*			
Root N Uptake	0.756*	p.714*	0.648*	0.961[a]	0.747*		
Total N Uptake	0.898**	0.939**	0.885[a]	0.851[a]	0.982[a]	0.859[a]	
Soil ppm N	0.217	0.217	0.161	0.125	0.322	0.123	0.202

*Correlated at P = .01 level.
**Correlated at P = .05 level.

[a]Values correlated with themselves.

analysis gave an "r" value of 0.85 indicating good agreement (Table 10). However, the values obtained for nitrogen recovery as measured by labelled nitrogen were not significantly different probably due to the low degrees of freedom (8) for the error term used for testing significance.

3. Soil Nitrogen

Inorganic soil nitrogen levels from the fresh *A. filiculoides* treatment were significantly higher than from the urea treatment (Table 10). No significant differences in inorganic soil nitrogen levels were observed between the four green manure treatments. This indicates that the addition of a green manure may increase the level of mineralized as well as mineralizable nitrogen available to the next crop.

4. Comparisons between Azolla and Leucaena

Leucaena was superior to all azolla treatments in terms of yields and equal to or better than the azolla treatments in terms of nitrogen uptake. Shih et al. (1978) obtained higher yields and nitrogen uptake from the incorporation of the legume, *Astragalus*, as compared to the incorporation of azolla. Since the azolla had a lower C/N ratio than the legumes used in both the experiments reported by Shih et al. (1978) and in the experiment reported herein, results obtained are due to some other factor than differences in the C/N ratio. Shih et al. (1978) suggested that the relatively slow rate of azolla mineralization may be due to the high lignin content of the azolla.

5. Effect of Rice Nursery Bed Fertilization

Recent studies by Kim (1978) showed that young rice seedlings absorbed fertilizer nitrogen shortly after germination and that nitrogen taken up before transplanting was used for plant growth after transplanting. The no nitrogen treatment using rice seedlings grown on azolla compost was designed to determine if azolla compost was sufficiently nitrogen rich to supply rice seedlings with a significant portion of the nitrogen needed for later growth.

The seedlings grown in azolla compost exhibited greater chlorosis at time of transplant than seedlings grown in tap water. The reasons for this chlorosis are not known. No significant differences in either yield or nitrogen uptake were obtained between this treatment and the zero nitrogen control treatment. The effectiveness of azolla or azolla compost as a nursery bed fertilizer for rice should be reexamined under conditions where nitrogen uptake by the rice seedlings is cearly not confounded by deficiencies in other necessary nutrients.

CHAPTER VI

GENERAL DISCUSSION

A. Fresh versus Air Dried Azolla

Results from the incubation experiment clearly indicates that air dried azolla is mineralized at a significantly slower rate than fresh azolla. The slower rate of mineralization for the air dried azolla is reflected in the significantly lower rice yields and nitrogen uptake levels obtained when air dried *A. filiculoides* was compared with fresh *A. filiculoides* in the greenhouse experiments. Yield and nitrogen uptake values for plants receiving an incorporation of fresh *A. filiculoides* were usually greater or showed no significant difference from those of plants fertilized with equivalent levels of nitrogen as with urea. However, yield and nitrogen uptake values for the air dried *A. filiculoides* treatments were consistently lower than those of the urea treatments.

Talley and Rains (1980), in their recent article, suggested that azolla used as a nitrogen source at low levels of nitrogen application is as available as ammonical nitrogen, while at relatively high rates of nitrogen fertilization azolla is considerably less available in terms of yields and nitrogen uptake than ammonical nitrogen.

However, the data upon which they based their conclusion was confounded by whether the incorporated azolla was fresh or air dried. The studies (Singh, 1977; Talley et al., 1977) cited by Talley and Rains (1980) which were conducted at low levels of nitrogen fertilization compared ammonical nitrogen to fresh azolla while the two studies conducted at high nitrogen input rates (Watanabe et al., 1977; Talley and Rains, 1980) used air dried azolla. Since the greenhouse experiments conducted for this research used application rates comparable to those employed by Talley and Rains (1980), the decreased availability of azolla found by them was probably due to their use of azolla as an air dried material rather than as a fresh material. Further field studies using fresh and air dried azolla at high nitrogen application rates need to be undertaken before definite conclusions can be drawn.

B. Effect of Azolla Dual Culture

Rains and Talley (1978) reported substantial increases in rice yields when *A. mexicana* was used in dual culture as compared to *A. filiculoides*. They suggested this resulted from ammonia being exuded from the growing *A. mexicana* but not from the growing *A. filiculoides*. When the azolla was being propagated for these experiments, it was noticed that under ambient greenhouse light conditions the growth of *A. mexicana* was inhibited by green algal contaminants.

However, when the *A. mexicana* was grown under a 56 percent shade cloth, little or no green algal contamination was observed and growth rates for the *A. mexicana* were increased. No green algae were observed growing with the *A. filiculoides* under either ambient light or shaded conditions. These observations suggest taht ammonical nitrogen was released to the flood water by the *A. mexicana* under high light conditions since nitrogen in the water would encourage algal growth.

In these experiments, the azolla was grown under 56 percent shade cloth to counteract the detrimental effects of high light intensities and temperature on azolla growth. Although vegetative growth of the azolla was increased due to shading, the turnover rate of the azolla appeared to be quite slow. It is unlikely that the dual culture of azolla of either species provided a measurable addition to the soil organic nitrogen pool during the course of rice growth. However, since a treatment involving azolla incorporation without dual culture was not included in the experimental design, it is not possible to determine the effect of the azolla dual culture on rice growth and nitrogen uptake.

C. Chemical Composition of the Organic Matter Incorporated

As mentioned previously, the significantly lower level of nitrogen availability obtained from the use of fresh *A. mexicana* compared to *A. filiculoides* was probably due to the much higher root to frond ratio of the former treatment. Similarly, leucaena treatments resulted in greater nitrogen availability than the azolla treatments. Recent studies by Shi et al. (1980) showed that humification coefficients correlated significantly with the lignin content of plant materials. They found lower rates of mineralization and higher levels of lignins in azolla than in either astragalus or water hyacinth. Rates of azolla mineralization were highest when the azolla was grown under optimal nutrients conditions and the azolla contained relatively low levels of lignin.

D. Transformation of Azolla Nitrogen

Results from the incubation experiments indicate that the incorporation of azolla into a flooded soil stimulates a flush of microbial activity within the first two weeks of incubation. Similar results were reported by Brotonegoro and Abulkadir (1977) under 60 percent water holding capacity soil conditions. Shortly after this flush of microbial activity, elevated levels of mineralized nitrogen were obtained. Within four weeks approximately half of the azolla nitrogen had been mineralized.

When fresh A. filiculoides was used as a green manure in greenhouse studies, 50 percent to 60 percent of the nitrogen added as azolla was recovered by plant uptake after 75 days of rice growth. At the same time the amount of soil inorganic nitrogen present was considerably higher for the azolla treatments than for the urea treatments. Long term experiments need to be conducted to determine the plant availability of residual nitrogen from azolla for crops following rice production.

E. Practical Use of Azolla as a Green Manure

In the greenhouse experiments conducted, nitrogen from fresh _A. filiculoides_ was shown to be as available as urea on the basis of both yields and nitrogen uptake. Significantly higher levels of residual inorganic soil nitrogen were found when fresh _A. filiculoides_ was incorporated into the soil compared to urea. This indicates that the incorporation of azolla may furnish nitrogen not only to the subsequent crop but also to later crops.

Long term increases in organic matter content have also been reported from the use of azolla (FAO, 1977). Since the organic carbon and total nitrogen content of the Hanalei soil used were very high, the percentage increase in soil organic matter parameters due to a single incorporation of azolla probably were small. Thus, measurements of total nitrogen and organic carbon at time of harvest were not performed.

Air dried _A. filiculoides_ and fresh _A. mexicana_, an azolla species with a high root to frond ratio, showed slower rates of nitrogen mineralization than were obtained from the use of fresh _A. filiculoides_. Results from this experiment indicated that well grown fresh _A. filiculoides_ can be as effective in increasing rice yields as urea. Rains and Talley (1980) indicated that air dried azolla can be used to supply at least half of the nitrogen required to obtain commercial levels of rice production in California. The results from both their studies and the experiments reported herein indicate that if air dried or a slow decomposing species of azolla are used as a nitrogen source, it may be necessary to use supplemental inorganic nitrogen fertilizers to obtain maximum rice yields.

The use of azolla as a green manure for rice growth will probably be based on economic and political factors as much as on agronomic factors. Important factors to be considered when using azolla are: (1) field time lost during the period of continuous flooding needed for azolla growth; (2) availability of land for use as an azolla nursery, which would permit azolla inoculation at high rates to the rice fields thereby minimizing the time required for azolla to multiply in the field; and (3) availability of labor for the incorporation of azolla into the soil. On the other hand the important factors to consider when using nitrogenous fertilizers are: (1) the energy, obtained primarily from non-renewable resources, required to produce these inorganic nitrogenous compounds via the Haber-Bosh reaction; (2) the availability of capital funds necessary for the purchase of these fertilizers; and (3) the reliability of transportation and distribution channels to provide those fertilizers where and when they are needed. In areas where either capital availability is low or long term agronomic benefits are preferred to short term economic gains, azolla will probably achieve its widest use.

CHAPTER VII

SUMMARY

An incubation experiment compared the CO_2 evolution and rate of nitrogen mineralization from the incorporation of whole fresh azolla, whole air dried azolla, fresh azolla fronds, and fresh azolla roots. Rates of nitrogen mineralization were generally greater due to the incorporation of whole fresh azolla or fresh azolla fronds compared to the incorporation of air dried azolla or fresh azolla roots. By the eighth week of incubation the amount of nitrogen mineralized from the whole air dried azolla was not significantly different from that obtained from the whole fresh azolla. Significantly higher levels of carbon mineralization were obtained from the incorporation of whole fresh azolla and fresh azolla fronds compared to whole air dried azolla and fresh azolla roots. No consistent significant differences in the rate of CO_2 evolution were observed between azolla treatments. However, the method used for the analysis of microbial activity probably did not give accurate results.

Two greenhouse experiments were undertaken to compare the availability of azolla nitrogen with that of urea. Measurements of straw and grain yield, nitrogen uptake by

the rice plant and residual levels of soil inorganic nitrogen were performed.

In the first greenhouse experiment a series of treatments was subjected to intermittent flooding. Yields from these treatments were significantly lower than from corresponding treatments under continuous flooding whereas nitrogen uptake levels were not significantly different. No significant differences in yield were obtained between the no nitrogen, urea, and azolla treatments under intermittent flooding. Higher rates of nitrogen application may be necessary if the detrimental effects of water deficiencies are to be at least partially alleviated.

In both greenhouse experiments, fresh *A. filiculoides* treatments generally produced yields that were not significantly different from urea treatments. In the first greenhouse experiment, nitrogen uptake by the rice plant was not significantly different when equal rates of nitrogen as fresh *A. filiculoides* and urea were compared. In the second greenhouse experiment, significantly higher nitrogen uptake levels were obtained from plants receiving nitrogen from the incorporation of fresh *A. filiculoides* compared to those fertilized with urea. In both experiments residual levels of inorganic soil nitrogen were significantly higher for the fresh *A. filiculoides* treatments compared to the urea treatments.

Use of *A. filiculoides* as an air dried material consistently resulted in yields and nitrogen uptake levels lower than those obtained when either fresh *A. filiculoides* or urea were used. In the second greenhouse experiment, the green manure *Leucaena leucocephala* significantly outyielded the azolla treatments but produced nitrogen uptake levels not significantly different from the fresh azolla. A comparison between fresh *A. mexicana* and fresh *A. filiculoides* showed no differences in rice yields but significantly higher levels of nitrogen uptake for the fresh *A. filiculoides* treatment. Results of nitrogen uptake due to added azolla obtained from labelled nitrogen transfer study were highly correlated with chemical nitrogen uptake analyses.

Based on the above results, fresh *A. filiculoides* was determined to be equally effective as ammonical nitrogen in increasing yields and nitrogen uptake of paddy rice under continuous flooding. If air dried azolla is used, supplemental inorganic nitrogen fertilizers may be necessary to obtain maximum yields.

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